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Qualitative analysis of carbohydrate contents of mycorrhizal mycelia after feeding of interconnected host plants with $^{14}CO_2$

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INTRODUCTION

It is recognized that inorganic ions are transported through mycorrhizal mycelium towards the host plant (HARLEY & SMITH, 1983). Carbon compounds are transferred to the fungus from the host root and transported, in the opposite direction, towards the growing hyphal tips. One fungal mycelium may establish mycorrhizas with severeal adjacent plant individuals and the resulting hyphal interconnections thus provide a potential pathway for the translocation of both inorganic and organic nutrients between host plants. Recent studies have shown that radioactive carbon is transported between mycorrhizal plants in this manner (BROWNLEE *et al.*, 1983; FRANCIS & READ 1984; READ *et al.*, 1985). In the present study composition of carbohydrates occurring in the extramatrical mycelium of ecto and ericoid mycorrhizal systems has been analysed and we have identified those carbohydrates which become labelled after feeding interconnected host plants with $^{14}CO_2$.

METHODS

Ectomycorrhizal systems. Ectomycorrhizas were first synthesized aseptically using *Pinus sylvestris* or *P. contorta* as host plants and *Suillus bovinus* or *Pisolithus tinctorius* as fungal associates. Infected plants were then transferred to perspex growth chambers (BROWNLEE *et al.*, 1983) containing peat in the case of *S. bovinus* association, or colliery spoil in the case of the *P. tinctorius* association. After mycorrhizal mycelia had grown from the infected roots to colonize the whole of the peat or spoil, uninfected host seedlings were introduced into the chambers. These rapidly became infected so giving rise to numerous mycelial interconnections between the plants. Between 50-120 µCi of $^{14}CO_2$, dependent on the chamber size, was now fed to one plant in each chamber and the systems were incubated in the growth room for 5-7 days. The chambers were then opened and fungal strands and mycelium were carefully detached from the substrates using fine forceps. The samples were immediately extracted with hot 80% ethanol and the ethanolic extracts were analysed by descending one way paper chromatography using ethyl acetate:pyridine:water

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(8:2:1) and ethyl acetate:acetic acid:water (14:3:3) as solvents. The chromatograms were scanned in a radiochromatogram scanner and the relative proportions of identifiable carbohydrates were calculated.

Ericoid mycorrhizal systems. Ten plants of *Vaccinium macrocarpon* were grown aseptically on a raft of filter paper, which was inoculated with *Pezizella ericae*, suspended over a nutrient medium so that the solution would rise by capillary action to the seedling roots. When mycorrhizal infection had become well established one of the plants was fed with 150 μCi $^{14}CO_2$ and the chamber was incubated in a growth room for 5 days. The plants and roots were then removed and the carbohydrates in the residual ericoid mycelium were extracted by washing the filter paper in hot 80% ethanol.

Parallel series of chambers, not exposed to $^{14}CO_2$, were employed for the analysis of total non-polar soluble carbohydrate contents. All extracts were de-proteinized using $Al(OH)_3$ and deionized. The carbohydrates were converted to silyl (TMS) ethers and separated on a column of 2% SE52 on Diatome 'C' (85-100 mesh) in a Pye 104 FID gas chromatograph using a temperature program of 140°C +4° min^{-1} to 290°C or 100°C + 4° min^{-1} to 290°C.

RESULTS

The results are summarized in Table 1. In *P. tinctorius* arabitol was the predominant carbohydrate followed by trehalose. The same pattern was found among labelled sugars. The GLC analysis of *S. bovinus* extracts showed trehalose to be the most important carbohydrate with arabitol, mannitol and sucrose each being found at a concentration equal to approximately one fifth that of trehalose. Of the labelled carbohydrates, mannitol was the most important, arabitol and trehalose being found in somewhat lower concentrations. Significant amounts of glucose were also found.

Extracts of *P. ericae* contained high levels of mannitol and trehalose together with some arabitol and sucrose. Mannitol, trehalose and sucrose were detected in extracts of labelled mycelium. In this case it is possible that both sucrose and mannitol could have originated from plant roots, some of which were inevitably left on the filter paper.

CONCLUSIONS

The major carbohydrates translocated through the external mycelium of the two mycorrhizal types were trehalose, arabitol and mannitol. Other carbohydrates were present but in relative small amounts. These compounds are recognized as being important fungal sugars and have been shown to be present both in the mycelial systems of non-mycorrhizal fungi like *Serpula lacrimans* (BROWNLEE & JENNINGS, 1981) and *Armillaria mellea* (GRANLUND *et al.*, 1985) and in the sheaths of ectomycorrhizal roots of beech (LEWIS & HARLEY, 1965). The major carbohydrates occurred in similar relative proportions in the radio-labelled extracts analysed by paper chromatography as in the non-labelled extracts analysed by GLC. However the relative importance of fungal sugars was very different in the different fungal species, which suggests that the spectrum of compounds moving between plants through mycelial interconnections will be to some extent dependent upon the identity of the fungal symbiont.

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Table 1. Relative amounts of carbohydrates in fungal strands or mycelium in three different mycorrhizal associations as determined by gas liquid chromatography (GLC) and by scanning of paper and radio chromatograms after feeding one host plant with $^{14}CO_2$. Trehalose was set to 100.

	Pisolithus tinctorius		*Suillus bovinus*		*Pezizella erica*	
	GLC	^{14}C scanning	GLC	^{14}C scanning	GLC	^{14}C scanning
Arabitol	466	250	20	160	5	-
Mannitol	28	13	21	200	130	240
Trehalose	100	100	100	100	100	100
Erythritol	N.D.	-*	-	-	-	-
Fructose	1	-	-	-	-	-
Glucose	37	-	-	50	-	-
Glycerol	N.D.*	N.D.	-	-	-	-
Sucrose	22	Trace	18	Trace	10	20

*N.D. Not determined
- Not detected